May 2, 1950.

Dr. L. L. Cavalli,
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England.

Dear Cavalli:

The cultures you requested are being sent under separate cover.

Like yourself, I had given up hope of an entirely satisfactory conclusion on 123 nutrition. Methionine is clearly essential, but some balanced combination which will be very difficult to reconstruct, but is available in protein hydrolysates, is necessary for prompt growth. I thought that methionine, threonine, and isoleucine plus valine might be the nearest approach to defining its nutrition, but the combination was far from satisfactory.

I am glad to hear that you have overcome your mysterious difficulties with recombination. We have never had anything like it!

We had once noticed some very minute, very motile elements in cultures of K-12 under phase contrast, but all I could see was the flash, and could not study them! We have not followed up this incidental observation. I hope you don't have the impression that we have espoused the idea of a microgamete. The question seems to be absolutely open. I suggested to Davis that he try a filter which would leak a few bacteria, in his experiment, but don't know whether he will follow it up.

You refer to some new crossing strains, in addition to 1113, etc. a) Does this mean that you confirm the fertility of W-1113? I have had such irregular results that I haven't been able to draw any definite conclusion. b) Do you have some new crossable strains? If so, can you tell me something of their history? I am very sorry that you were unable to correct your Nature note in re Tatum. Professor Tatum played an original and direct role in the early work, but has been kind enough to leave much of its later development to me. Through no fault of mine, I hope, he has not been adequately recognized for it. He would be the last person in the world to make any fuss about it, but may I suggest that it might be appropriate to make a marginal correction on any offprints that you may send him.

As you may imagine, I have been groping for almost two years for a chromosome-aberration interpretation of the segregation data! Any number of hypotheses have come up, but unfortunately very few could be tested with the experimental material available. I am reasonably thoroughly convinced of linearity on the basis of M V6 Lac V1 TL data. One critical stumbling block has been that two viable segregants from a single heterozygous diploid, when intercrossed, have given precisely the same aberrant behavior as the diploid itself, or its parents. This seems to rule out one of the most attractive explanations I had thence formulated: that 677 and 58-161 differed in chromosome structure, in respect to a reciprocal translocation between <u>two</u> chromosomes. The hemizygous diploids would then have represented 3-1 segregations of the synaptic complex. However, as I may have mentioned, some segregants from diploids have been found to show

unique linkages in subsequent outcrossings, along the same lines as your evidence from W-705. With regard to the latter, however, I would raise the question whether the presence of both sets of selected markers (viz BM and MlyTr in the same region [right of BM] as Lac might impose an apparent linkage of greater intensity that the smaller, difficult to interpret, interaction that both Newcombe and I have found between Gal ahd Lac. Or, is it possible that, e.g., Mly may be linked to Lac, and Tr to Gal, and that both Mly+ and Tr+ segregate in low proportion for the same reason as Mal^{I+}?

Another complication has come up in the analysis of segregation. In a Lacv Malv diploid, H-226, I have found "partial" segregants: e.g. Lacv Mal-. But unlike the Lac v Mal- usually picked up in Het crosses, these pure Mal- are homozygous for Mal, giving Malv when reversions arecselected on EMS maltose. H-226 is also segregating for Xyl, and I found one Lacv Xylv Mal-. The linkage of Xyl to Mal allows two types of Mal v reversions to be distinguished: Xyl+ Mal+/Xyl- Mal- (coupling, or "cis"), and + - / - + (repulsion or "trans2). Here at last one should find a distribution into two equal classes, uhless there are effects on the mutability of the two Mal- alleles. 10 have so far been tested: 6 cis; 4 trans. I hope to test enough to come to some more decisive conclusion as to the equality of the two types, as I think that this may be a fundamental, though not a pregnant, datum supporting the whole concept of chromosomal organizatic Also found as partial segregants: Lac- Malv Xyl v; Lacv Mal- Xyl-, and others. Especially because of the latter, I don't think we are dealing with spontaneous, [and very frequent] mutation from Mal+ to -. At any rate, these "partial segregatic would appear to result from some sort of meiotic nondisjunction or refusion process. They parallel the process by which Het crosses yield diploids homozygous for factors distinct in the parents. But so far, I have not found any parallel in the behavior of a persistent diploid for the elimination, e.g.of Mal, that takes place in the usual formation of persistent diploids. [Mr. Gordon Allen, working with Davis, has some new data confirming the notion that this elimination also occurs in production of "typical" prototrophs— he has been finding the complementary types, e.g. P-T- and M-L- in nutritional selections from M-P- x T-L-. The complementary types are not complementary for Mal.] We have then to think of the following peturbations: a)deletions, usually for Mal, etc., either from the gametes or the zygote at an early stage, b) probably repeated (how many times?) meiosis with refusions of meiotic products [or non-disjunction], c) possibly a consequence of a, non-random segregation of factors. I am hopeful that this can all be explained on a chromosome structural basis, bu am inclined to think in terms of aberrations (rings; duplications) that do not require structural heterozygosity to give interesting results.

There was very little of general biometric interest in my thesis, and I don't think it would be worth the rouble to get it. The problem of estimating the abolute map distance from triple-crossover frequency was handled as follows:

BM a Lac b Vl c Tl a+b+c = x = map distance

Since there is assumed to be no interference, there will be a Poisson distribution of crossovers in x, or in any part a.... Let $r_a \ldots r_d$ represent the proportions of prototrophs found in each of the 3 single crossover classes, or the triple class. Since odd numbers of crossovers are needed for a recombination, we sum the odd terms of the Poisson series, to give., e.g. $e^{-x} \sinh x \; [= 1/2 \; (1-e^{-2x})]$.

We thus can obtain expressions of the form:

$r_a = (\sinh a)(\cosh b)(\cosh c) / \sinh x$ and

$r_d = \sinh a \sinh b \sinh c / \sinh x$. From this, one obtains explicit solutions of the form:

$$\tanh^2 a = r_a r_d / r_b r_c. \qquad a = \tanh^{-1} (r_a r_d / r_b r_c)^{1/2}$$

If the contributions of multiple crossovers to r_a, etc., can be ighored, the simpler approximation:

$r_d = \sinh s_a x . \sinh s_b x . \sinh s_c x \; / \sinh x$ is derived which has the advantage of being a single equation in one variable, x.

(where $s_a = r_a / 1-r_d$)

Sincerely,

Joshua Lederberg